The WellPro Automated
Liquid Handling System tm

ProGroup

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WellPro 96 and 384 Liquid Handler

User Manual

User Manual Rev. 2.0; November 2004

NOTE: This manual is written to be used for either the WellPro 96 or WellPro 384 instruments. The instruments vary in that the liquid heads are specifically designed for either a 96 or 384 well microplate. The WellPro 96 uses a 12 channel liquid head with 200ul capacity tips, and the WellPro 384 uses a 24 channel liquid head with 50ul capacity tips.

The examples given in the text have been written for the WellPro 384 ie; Rows A to P, Columns 1 to 24, transfer volumes 50ul max, etc. When using the WellPro 96 the appropriate volumes and positions will be displayed on the hand controller. The tutorial examples given can be followed for either instrument. WellPro 96 specs are included on this CD-ROM under the WellPro 96 Brochure. If you prefer a hard copy of this manual exclusively for the WellPro 96 please contact Customer Service at 1-800-471-1916, 1-618-258-7393, or www.wellpro.us

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Printed in the United States

SAFETY SYMBOLS AND MARKINGS

These symbols are intended to draw your attention to essential information and alert you to the presence of hazards as indicated. Some of these symbols may not appear in the manual or on the product:

SAFETY SYMBOLS used in the WellPro Model 384 Liquid Handler

Power ON

Power OFF

WARNING MARKINGS used in the documentation

Caution: risk of electric shock.

Caution: biohazard risk.

Caution: risk of personal injury to the operator or a safety hazard to the surrounding area.

Caution: risk of damage to the instrument, other equipment or loss of performance or function in a specific application.

ABOUT THE USER MANUAL

This User Manual has been written for the user (e.g. laboratory technician) and provides information on the WellPro Model 384 Liquid Handler. This manual contains the installation and operating instructions for the WellPro Model 384 Liquid Handler. **Read the manual in its entirety prior to operating the instrument.**

This User Manual has been designed to give you the information you need to:

- Review safety precautions.
- Install the WellPro Model 384 Liquid Handler.
- Use the WellPro Model 384 Liquid Handler in routine analyses and research.
- Perform basic maintenance procedures.

This User Manual also describes features and specifications of the WellPro Model 384 Liquid Handler hardware and on-board software.

TABLE OF CONTENTS

SAFETY SYMBOLS AND MARKINGS III

ABOUT THE USER MANUAL IV

TABLE OF CONTENTS V

1 INTRODUCTION 1

1.1 OVERVIEW 1
1.2 PRINCIPLE OF OPERATION 2
1.2.1 Controller 2
1.2.2 Liquid Handling Unit 2
1.2.3 WellPro Reagent Containers 2
1.2.4 WellPro Templates 3
1.3 ABOUT THE USER MANUAL 4

2 INSTALLATION PROCEDURES 5

2.1 OVERVIEW 5
2.2 LOCATION OF THE UNIT IN THE LABORATORY 5
2.3 POWER REQUIREMENTS 5
2.4 UNPACKING AND INSPECTION 6
2.5 INITIAL INSTALLATION OF THE WELLPRO 384 LIQUID HANDLER 6

3 SYSTEM COMPONENTS 7

3.1 INTRODUCTION 7
3.2 WELLPRO WP384 CONTROLLER 7
3.3 THE WELLPRO WP384 LIQUID HANDLING UNIT 7
3.4 THE WELLPRO REAGENT CONTAINER 7

4 ESTABLISHING A FILE USING THE CONTROLLER 8

4.1 INTRODUCTION 8
4.2 THE KEYPAD CONTROLLER 8
4.2.1 The Display 8
4.2.2 The Keypad 9
4.2.3 Clear 9
4.2.4 Start 9
4.2.5 Step 10
4.2.6 Stop 10
4.2.7 Help 10
4.2.8 File 10
4.2.9 Yes/No 11
4.2.10 Enter 11
4.3 ENTERING DATA INTO A FILE 11
4.4 FILE 0 11

5 PLATE FILLING 12

5.1 OVERVIEW 12
5.2 EDITING A PLATE FILLING FILE 12
5.3 STORING/OVERWRITING THE FILE 16
5.4 RUNNING A PLATE FILLING FILE 16
5.5 SAMPLE PLATE FILLING EXPERIMENT 17

6 SERIAL DILUTION 19
6.1 OVERVIEW 19
6.2 EDITING A SERIAL DILUTION FILE 19
6.3 STORING/OVERWRITING THE FILE 23
6.4 RUNNING A SERIAL DILUTION FILE 23
6.5 SAMPLE SERIAL DILUTION EXPERIMENT 24
7 PLATE TO PLATE TRANSFER 26
7.1 OVERVIEW 26
7.2 TYPES OF PLATE TO PLATE TRANSFER 26
7.3 EDITING A PLATE TO PLATE TRANSFER FILE 27
7.4 STORING/OVERWRITING THE FILE 31
7.5 RUNNING A PLATE TO PLATE TRANSFER 31
7.6 SAMPLE SERIAL DILUTION EXPERIMENTS 32
8 EMPTYING A PLATE 36
8.1 OVERVIEW 36
8.2 EDITING AN EMPTYING A PLATE FILE 36
8.3 STORING/OVERWRITING THE FILE 39
8.4 RUNNING AN EMPTY A PLATE FILE 39
9 TIME DELAY FILE 40
9.1 OVERVIEW 40
9.2 EDITING A TIME DELAY FILE 40
9.3 STORING/OVERWRITING THE FILE 42
9.4 USING A TIME DELAY FILE 42
10 LINKING FILES 43
10.1 OVERVIEW 43
10.2 USING LINKED FILES 43
10.3 A TYPICAL SERIES OF OPERATION USING LINKED FILES 43
11 ADJUSTING THE TIP HEIGHT 45
11.1 OVERVIEW 45
11.1.1 Editing/Running the Tip Height Adjustment File 45
11.1.2 Setting the Tip Height 46
12 CALIBRATION PROCEDURES 49
12.1 INTRODUCTION 49
12.2 THE GRAVIMETRIC METHOD 49
12.2.1 Materials Required 50
12.3 CALIBRATION PROCEDURE 51
12.4 CALCULATIONS 53
12.5 CALIBRATION OF NON-AQUEOUS OR VISCOUS SOLUTIONS 55
12.5.1 Introduction 55
12.5.2 Procedure 55
12.6 QUALITY CONTROL PROCEDURES 57
12.6.1 Verification 58

13 OPERATING HINTS AND MAINTENANCE 59
13.1 OVERVIEW 59
13.2 TIPS 59
13.3 POWER INTERRUPTIONS 59
13.4 MAINTENANCE 60
13.4.1 Preventive Maintenance 60
13.4.2 Cleaning up Spills 60
13.4.3 Piston Plungers 60
13.4.4 Table Rails 60
13.4.5 Cleaning the Plastic Cover 60
13.4.6 Fuse Replacement 61
13.5 TROUBLESHOOTING 61
14 WELLPRO WP384 SPECIFICATIONS 62

1 INTRODUCTION

1.1 Overview

The ProGroup WP384 Liquid Handler (Figure 1.1) is designed to perform the following liquid handling activities on a user-programmable basis:

- Precisely transfer liquids in volumes from 1 µL to 50 µL using disposable tips
- Perform serial dilutions in almost any ratio,
- Perform serial dilutions of 16 wells or 24 wells simultaneously
- Precisely mix the contents of a microplate
- Transfer samples to or from microplates
- Perform mother-daughter "repli-plating"
- Add a reagent to the wells on a plate at a user specified time

Fig 1.1 WellPro WP384 Liquid Handler

1.2 Principle of Operation

The WellPro WP384 Liquid Handler consists of a hand-held controller and a liquid handling unit. A variety of reagent containers can be used to hold liquids that will be added to the cells in a plate (or have been removed from a plate).

1.2.1 Controller

The WellPro WP384 Liquid Handler includes a keyboard and display that are used to program all operations of the unit. The controller allows the user to control parameters such as the volumes to transfer, mix, or dispense, the transfer speed, and whether to change tips between transfers.

1.2.2 Liquid Handling Unit

The WellPro WP384 Liquid Handler is an electro-mechanical transfer unit that is comprised of two basic parts: a table and a vertical head assembly.

The *table* accommodates a series of interchangeable templates which position the reagent containers and a magazine with the disposable tips, etc. During operation, the table slides back and forth to bring the appropriate component under the head assembly.

The *head assembly* consists of three basic parts:

- a plunger housing which contains nozzles that press into the tips to form airtight seals
- a plunger block which moves up and down to aspirate and deliver samples
- a tip ejector plate which removes used tips

The head assembly slides up and down to bring the tips to the appropriate height to perform the designated operations.

In most instances, a 24 channel (tip) air displacement pipetting head is used on the WellPro WP384 Liquid Handler and most protocols are designed to be performed in the 24 channel mode, but serial dilutions can be performed in the 16 channel mode to allow for maximum dilution capability with the 384 well plate.

1.2.3 WellPro Reagent Containers

The WellPro liquid handler can be used with a variety of MICROTROF reagent containers, each of which consists of three reservoirs. Each reservoir holds a different amount of liquid: (e.g. 20 mL, 58 mL, and 77 mL). You can use the reservoirs to hold reagents that will be added to a plate or waste that has been removed from a plate. In addition, some MICROTROFs include a station for storage of microtips.

1.2.4 WellPro Templates

The WellPro WP384 liquid handler uses a template to position microplates, reagent containers and tip magazines on the movable table.

Two templates are provided:

- **Standard Template (24 Across)**

  A general purpose template (Template 1 - "24 Across") is used for liquid handling operations when the 24 well rows on the plate are oriented across the front of the instrument as shown in Figure 1.2.

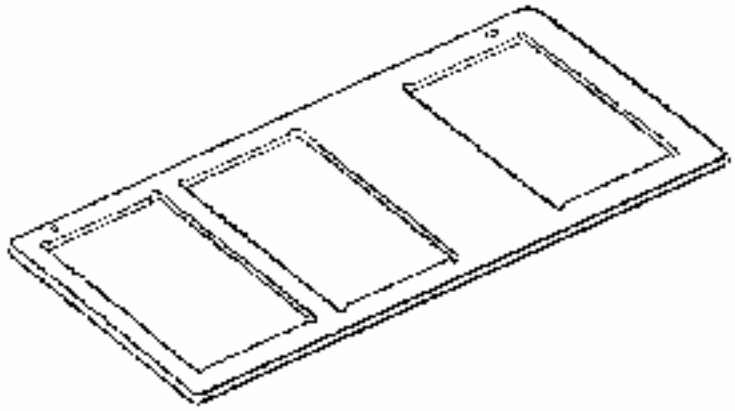

Fig 1.2 Standard Template

- **Serial Dilution Template (16 Across)**

  The Serial Dilution template "16 Across") is used when serial dilution is to be performed lengthwise on a plate. In this case, the plate is oriented such that the 16 wells of a column face the front of the instrument. (Figure 1.3).

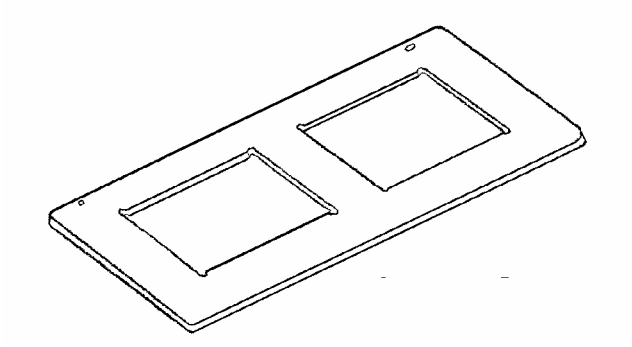

Fig. 1.3 Serial Dilution Template

1.3 About the User Manual

This User Manual has been written for the end user of the system (e.g. a laboratory technician) and provides information about the operation and maintenance of the WellPro WP384 Liquid Handler.

Read the manual in its entirety prior to operating the instrument.

This User Manual has been designed to give you the information you need to:

- Review safety precautions.
- Install the system
- Use the system in routine jobs and research.
- Perform basic maintenance procedures.

This User Manual also describes features and specifications of the WellPro WP384 Liquid Handler.

This manual includes the following sections:

- Installation (Chapter 2)
- System Components (Chapter 3)
- Establishing a File (Chapter 4)
- Plate Filling (Chapter 5)
- Serial Dilution (Chapter 6)
- Plate to Plate Transfer (Chapter 7)
- Emptying a Plate (Chapter 8)
- Time Delay Files (Chapter 9)
- Linking Files (Chapter 10)
- Adjusting the Tip Height (Chapter 11)
- Calibration Procedures (Chapter 12)
- Operating Hints and Maintenance (Chapter 13)
- Specifications (Chapter 14)

In addition, a series of appendices include the warranty and ordering information.

2 INSTALLATION PROCEDURES

2.1 Overview

The WellPro 384 Liquid Handler is designed to be installed by the operator. This chapter describes how the unit is to be unpacked and assembled.

2.2 Location of the Unit in the Laboratory

The unit should be installed in a facility with the following conditions:

- The temperature should be maintained between 20-26◦C (68-79◦F). To prevent temperature fluctuations, do not place the WellPro 384 liquid handler in direct sunlight, near equipment that gives off heat, or under heating or air conditioning outlets.
- If flammable or toxic solvents are to be used, a suitable ventilation system should be provided.
- The use of open flames in the laboratory should be prohibited.
- Corrosive vapors or dust should not be present as these materials can adversely affect the long-term performance of the system.

The Well-Pro fluidics module requires approximately xx mm (yy") of linear bench space. In addition, the controller requires xx cm (yy").

Note: Place the WellPro 384 on a stable, level bench or hood. Excessive jarring or vibration during operation may affect its performance.

2.3 Power Requirements

The WellPro 384 Liquid Handler to designed to operate at 100, 120, 220 or 240 V (50/60 Hz) with a maximum power consumption of 360 watts. The operating potential is set at the factory and is indicated on the serial number tag on the rear panel of the system.

WARNING: Verify that this voltage is correct for your location before installation.

CAUTION: WellPro 384 Liquid Handlers shipped to locations in the United States and Canada are supplied with a standard three-conductor power cable and a three-terminal plug that provides an earth ground. The unit MUST be connected to a three-terminal socket that is properly wired according to the U.S. National Electrical Code and is grounded to a true earth ground. The use of an adapter to a two-terminal outlet is PROHIBITED.

2.4 Unpacking and Inspection

The WellPro 384 Liquid Handler is shipped in one carton that contains the items listed in Table 2.1. When the system is received, carefully unpack the unit and verify receipt of all components. We recommend that you save the shipping container and packing material in case you need to move or ship the instrument.

Table 2.1 PACKING LIST

Quantity	Description	Part Number
1	WellPro WP384 Automated Liquid Handler	
1	Controller	
1	Controller Cord	
1	Plastic Cover	
1	Operators Manual	
1	Microtrof	
1	Pipette Tip Rack	
1	Template - Horizontal Format (24 Across)	
1	Template - Vertical Format (16 Across)	

If there is external damage to the shipping carton, report the damage to the shipping company and your local representative. If internal parts are missing or damaged, report this to the shipping company and your local representative as soon as the problem is observed.

☞ **Note: If there us any apparent damage to the system, the user should investigate the nature of the damage before plugging the unit into the mains to ensure that powering up of the system will not create a hazardous condition or damage internal components. If the damage appears significant, call your local representative before connecting the unit to the mains.**

2.5 Initial Installation of the WellPro 384 Liquid Handler

Assembly, installation and final inspection of the WellPro 384 Liquid Handler will be done by the user. To install the unit:

1. Connect the Controller Cord to the Controller and the rear on the liquid handling unit.

2. Place the power cord to the line into the socket into the rear panel of the liquid handling unit and plug the unit into the line.

Power up the unit. The liquid handler will initialize and the controller will present Figure 2.1. The pipette tip will move to determine the zero. If the head does not move, contact your local service representative for assistance.

Figure 2.1: The Keypad Controller

Press the START button. The pipette tip will move in the X, Y and Z direction. If the head does not move, contact your local service representative for assistance.

3 SYSTEM COMPONENTS

3.1 Introduction

The WellPro 384 Liquid Handler system includes the following components:

- WellPro Controller (Section 3.2)
- WellPro WP384 Liquid Handling Unit (Section 3.3)
- WellPro Reagent Containers

3.2 WellPro WP384 Controller

The WellPro WP384 Controller includes a keyboard and display which provides the operator with complete control of all liquid handling operations. The basis of operation is via a user generated method which includes a series of primary operations. The method can be stored and retrieved as desired.

Each primary operations provides for user control of a given aspect of the operation. Typical operations include the volumes to transfer, mix, or dispense, the transfer speed, and whether to change tips between transfers.

3.3 The WellPro WP384 Liquid Handling Unit

The WellPro WP384 Liquid Handling Unit is an electro-mechanical transfer unit that includes a table and a vertical head assembly. The table accommodates interchangeable templates which position reagent containers, a magazine with rows of disposable tips, etc. During operation, the table slides back and forth horizontally to bring the appropriate workstation under the head.

The head assembly consists of three basic parts:

- A plunger housing which contains nozzles that press into the tips to form airtight seals
- A plunger block which moves up and down to aspirate and deliver samples,
- A tip ejector plate which removes used tips. The head assembly slides up and down vertically to bring the tips to the appropriate height to perform the designated operations.

A 24 channel (tip) air displacement pipetting head is included on the WellPro liquid handler as most protocols are designed to be performed in the 24 channel mode, but serial dilutions can be performed in the 16 channel mode to allow for maximum dilution capability with the 384 well plate.

The table assembly drives the plate which holds the well plates and the Microtrof reagent containers.

3.4 The WellPro Reagent Container

The WellPro liquid handler is used with a Microtrof which is a reagent container. The Microtrof container consists of up to three reservoirs, each of which is used to contain a reagent or solution. In some cases, fluid from the wells is deposited in the Microtrof.

The WellPro WP384 liquid handler uses templates to position microplates, reagent containers and tip magazines on the movable table. Two templates are provided as described in Section 1.3.4.

4 ESTABLISHING A FILE USING THE CONTROLLER

4.1 Introduction

The basis of operation of the WellPro 384 Liquid Handler is the *file*, which is a set of instructions and conditions that are used to perform the desired operation. The operator can establish, store and execute files via the controller module.

The system includes a number of template files that can be used to establish files for actual operation. To establish a file for a specific operation:

- Open the template file that corresponds to the action that you want to perform.
- Edit the method to meet the specific requirements of your operation.
- Save the file.

As an example; if you want establish a file to be used to add 20 µL to each well in a plate, open *File 61* (which is the template file for adding fluid to a plate), edit the file as appropriate and save it using a different file number (e.g. File 27). When you are ready to run the operation, simply recall the file and press **Start**. When a file is executed, the steps are performed in the indicated order.

In this chapter, we describe the keypad controller and explain the general approach to file editing. Discussion of the individual file templates is presented in Chapters 5-9.

4.2 The Keypad Controller

4.2.1 The Display

When the system is powered up, the display will be as shown in Figure 4.1. The nature of the information on the display will depend on the status of the system, as described below.

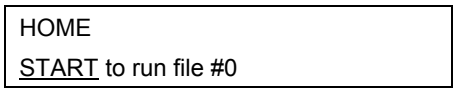

Figure 4.1: The Keypad Controller

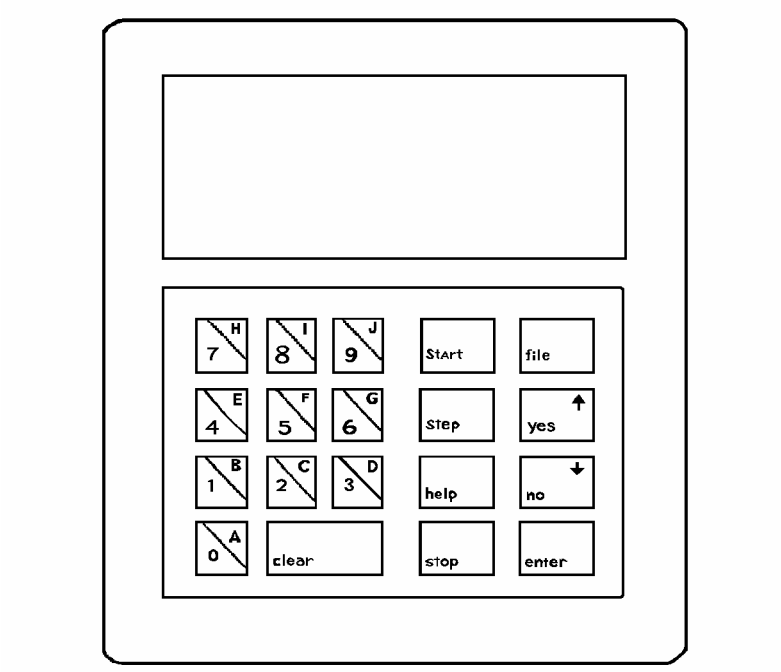

Figure 4.2 The Controller

4.2.2 The Keypad

The controller includes ten alphanumeric keys and nine control keys. The general role of the control keys is described below. For many keys, the action is dependent on the status of the program.

4.2.3 Clear

1. Removes information that you have entered on the display before it is entered into the WellPro controller. This key is most commonly to erase information and start over when you are editing a file.

2. Erases an entire user-created file by pressing the **File** key, the appropriate number keys, the **Enter** key, the **File** key and the **Clear** key in that order.

4.2.4 Start

1. Instructs the system to start to carry out the instructions and conditions in the active file.

2. Restarts a file that has been paused by the user before completion. The file will start where it left off when it was paused.

4.2.5 Step

The **Step** key has several uses:

- At the start of a file, **Step** indicates that you want to edit or review the file.
- While editing a file, **Step** is used to indicate that the present setting for the parameter is acceptable, and you want to see the next parameter.
- When the *End of File* message is displayed, start of the file will be presented for editing or review.
- After you have pressed **Stop** during the execution of a file, the activity indicated in the next line in the file will be performed.

4.2.6 Stop

The **Stop** Function has several uses:

- If you are editing a file, the END OF FILE message will be presented. At this point you have two choices: If you press **Enter** and the press **Start**, the file will be run. If you press **Stop** again, you will be able to enter a new file.
- If you are running a file, the WellPro liquid handler will pause (stop) the present operation. Once the WellPro liquid handler has paused, you may either abort the file by pressing the **Stop** key again), or go through the file one motion at a time by pressing the **Step**.
- If you are using the Tip Height File (File 70), the **Stop** key is also used to exit from the file
- If you are using the Home-Pump Prime File, the **Stop** key will stop priming the pump.

4.2.7 Help

1. **Help** is normally used to determine the numeric range for a key sequence.

2. During file editing, the Help key allows you to override the range limits on many parameters. Normally, the WellPro WP384 Liquid Handler will verify the validity of a parameter (e.g. the transfer volume) when it is entered by checking against the allowable range of values for the parameter (which is shown on the display). By pressing the "help" key, the controller in instructed to accept ANY entered value, subject only to physical limitations of the instrument.

4.2.8 File

A **File** is a stored set of instruction and conditions which the WellPro WP384 Liquid Handler uses to perform the desired activity. The user selects the desired file via the alphanumeric keypad and presses the **File** followed by the **Enter** key to load it. 70 files are available.

Files 51 through 70 are pre-programmed in WellPro WP384; and you can modify them to create new files. Files 1 through 45 are user-storage files. The initial standard files are as follows:

0	HOME-PUMP PRIME
61	PLATE FILLING
62	SERIAL DILUTION
63	PLATE TO PLATE
70	TIP HEIGHT

4.2.9 Yes/No

The **Yes** and **No** keys are used to respond to questions asked by the WellPro controller and tell it whether you want it to do certain tasks. Each option will be underlined in sequence until you press "step".

4.2.10 Enter

1. If the **Enter** key is pressed after an alphanumeric key has been selected, the indicated value is included in the file.

2. If the **Enter** key is pressed at the END OF FILE (RUN), the run will start.

4.3 Entering Data into a File

Each of the template files contains a series of steps that are used to enter the appropriate data for the task to be performed and you can scroll through the steps via the **Step** key. When you come to a step that must be edited, the appropriate selection can be made in one of two ways:

- *The user must enter a numeric value within the range.* As an example, the **VOLUME IN WELL AT START** step (Figure 4.3) is used to tell the liquid handler how much liquid is in each well before any transfer has been made. The WellPro uses this number to determine how far down into the wells the tips should go and also to prevent the instrument from attempting to add more liquid than each well can hold.

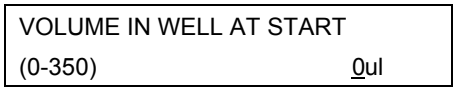

Fig 4.3 The Volume in Well at Start Step

  You can set the value of this parameter from 0 to 50 μL in 1 μL increments via the numeric keypad. After the desired value has been entered, press **Enter**.

****the range says 350?

- *The user must choose one of a set of discrete values or selections.* As an example, the **PLATE TYPE** step (Figure 4.4) is used to tell the WellPro liquid handler whether your plate has a FLAT, U-shaped, or V-shaped bottom. The WellPro liquid handler uses this information to determine the height of the liquid in each well and thus how far down into the wells the tips should go. One, and only one value is used for a given plate.

Fig 4.4 The Plate Height Step

To select the desired option, press the YES/↑ or NO/↓ key until it the desired type is highlighted, then press Enter.

4.4 File 0

File 0 (which is presented when the unit is powered up) is used to initialize the system.

5 PLATE FILLING

5.1 Overview

Plate filling refers to the addition of a user specified quantity of liquid to the wells in a plate. The template for plate filling is File 61, which is used to establish a user file which can be stored and used as desired (and can be as a template for additional plate filling files).

5.2 Editing a Plate Filling File

When you open file 61 (or any other plate filling file), the opening step is presented and the step command should be employed to view the file on a screen-by-screen basis. You can change the value of each parameter to provide the desired program to fill the plates. After a value is entered, press ENTER. To advance to the next step, press STEP. A detailed discussion of role of each key is presented in Chapter 4.

1. **OPENING STEP**

PLATE FILLING
STEP TO EDIT FILE # 61

When File 61 is opened, the **OPENING STEP** display is presented. If you want to view/edit the file, press STEP.

2. **PLATE POSITION STEP**

PLATE POSITION
1 - 2

PLATE POSITION is used to whether the plate to be filled is in the FRONT cavity of the template (Position 1), the second cavity of the template (Position 2) or both. Underline the desired position(s).

3. **VOLUME IN WELL AT START**

VOL IN WELL AT START	
(0-350)	0ul

VOLUME IN WELL AT START is used to indicate tell how much liquid is in each well before any transfer has been made. This number is used to determine how far down into the wells the tips should go and also to prevent the instrument from attempting to add more liquid than each well can hold. This parameter can be set from 0 to 50 µL in 1 µL increments.

4. **DELIVER FROM**

DELIVER FROM
T1 - T2 - T3

DELIVER FROM is used to indicate the MICROTROF reservoir that contains the liquid to be dispensed. T1, T2, T3 refers to the front, middle, and rear reservoirs of a MICROTROF container. Underline the desired choice.

5. **MIXES IN MICROTROF**

MIXES IN MICROTROF	
9	3

MIXES IN MICROTROF is used to specify mixing of the MICROTROF contents before an aliquot is taken. Mixing of a MICROTROF is especially important with cells or other inhomogeneous samples. This parameter can be set between 0 and 9 (0 indicates no mixing).

6. **MICROTROF MIX VOLUME**

MICROTROF MIX VOLUME	
(0-200)	50ul

MICROTROF MIX VOLUME is used to indicate how much liquid to aspirate into the tip and dispense back into the MICROTROF in order to mix the MICROTROF contents. This parameter can be set from 0-50 in 1 µl increments (0 indicates no mixing). Mix efficiency may vary with MICROTROF volume.

7. **DELIVERY VOLUME**

DELIVERY VOLUME	
(0-200)	50ul

DELIVERY VOLUME indicates how much solution should be dispensed into each well from the MICROTROF reservoir. This parameter can be set from 0-50 in 1 µL increments.

8. **MIXES AFTER TRANSFER**

MIXES AFTER TRANSFER	
(0-9)	2

MIXES AFTER TRANSFER is used to specify the number of mixes after the aliquot has been delivered to the plate. This parameter may be set from 0-9 in increments of 1 (0 indicates no mix after transfer).

9. **MIX VOLUME**

MIX VOLUME	
(0-50)	50ul

MIX VOLUME is used to specify the volume of the aspirate/delivery cycles used to mix the contents of the well after transfer. The upper limit is calculated as 75% of the transfer (fill) volume. This parameter can be set from 0 to the upper limit in increments of 1 µL.

10. **PLATE TYPE**

PLATE TYPE
FLAT-U-V-HEIGHT-TUBE

PLATE TYPE is used to indicate the design of the bottom of the plate. This information is used to determine the height of the liquid in each well and thus calculate how far down into the wells the tips should go. If you are not using a standard microplate, select HEIGHT and determine the amount to raise or lower the tip height by using File 70, the Tip Height File (Section 11). The height parameter can be set from 100 to 3500 in increments of 1.

11. FILL PLATE ROWS

FILL PLATE ROWS
A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P

FILL PLATE ROWS is used to indicate the rows of the plate to fill and which to leave unfilled. Rows are designated by letters A through P. To select row A, press YES. The underline will remain and B will be active (if you do not want to use a row, press NO to advance to the next row). If you want all rows to be filled, press the STEP key. After the last NO selection has been made, press STEP (i.e. if you want all rows except for A, press NO for A. The active row will become B. If you press STEP, rows B-P will be active.

12. MICROTROF VOLUME AT START

MICROTROF VOL AT START	
(20-80)	77ml

MICROTROF VOLUME AT START is used to indicate how much liquid is in the reservoir you have chosen before any liquid has been removed. The system uses the delivery volume and the number of rows to be filled to calculate the minimum volume needed to run the file and indicates it on the display as the lower limit. You can set the value of this parameter in 1 mL increments from this lower limit to 20 mL, 60 mL, or 80 mL for the front, middle, and rear reservoirs respectively for the MICROTROF reservoir.

After the file has been run, the display will update the value to reflect the decreased amount of liquid in the reservoir.

13. CHANGE TIPS

CHANGE TIPS? YES/NO	YES

CHANGE TIPS is used to indicate if the tips should be removed after the first liquid is dispensed and a new set of tips from the tip magazine should be placed on the pipette head, thus decreasing carryover. If you don't want to change tips, then the same tips will be used throughout the entire operation.

14. LIQUID HANDLING SPEED

LIQ HANDLING SPEEDS
RAPID-MEDIUM-SLOW

LIQUID HANDLING SPEEDS is used to select the rate sample delivery from the tips (rapid, medium, or slow).

15. LINK TO STORED FILE

LINK TO STORED FILE	
(0-45, 0=STOP)	0

LINK TO STORED FILE is used to indicate what the system should do after it completes the file it is currently running.

If you want the system to stop at the end of the file, enter 0.

If you want the system to perform another file after the present file is complete, enter the desired file number. The LINK TO STORED FILE command permits more efficient operation because you don't need to start each individual file manually with the "file", "##" A detailed discussion of Linked files is presented in Section 10.

16. END OF FILE

END OF FILE
RUN-STORE

END OF FILE is used to indicate what the system should do after you have completed the editing of the file:

- If you select RUN, the file will start.
- If you select STORE, the file will be copied into memory (see Section 5.3 for additional information about storing files).

Note: You can also review the file you have just made by continuing to press the STEP key.

17. ENTER FIRST TIP ROW

ENTER FIRST TIP ROW	
(A-P)	P

5.3 Storing/Overwriting the File

To store a new file:

1. At the END OF FILE statement (item p, Section 5.2), select STORE. The display will appear as shown below.

FILE STORE
ENTER USER # ____

2. Enter your user number or project number (this number is provided for reference only and does not affect the use of the system), then press ENTER. The display will indicate:

FILE STORE
ENTER FILE #XX

where xx is the file number assigned by the system (it will be the lowest unoccupied file). If you want to assign a different number, enter it.

3. Press ENTER. The display will present:

YES TO STORE
USER #zzzz FILE #XX

4. Press YES to accept the assignment.

5.4 Running a Plate Filling File

When you are ready to run a Plate Filling file:

1. Select the desired file

2. Press **START**

If the MICROTROF does not contain the required amount of liquid to perform the desired operation, the message **PLEASE FILL MICROTROF** will appear on the screen. This prompt allows you to simply enter the volume you are adding to the MICROTROF reservoir without requiring you to step back through each of the file's parameters.

At the conclusion of the operation, the system will stop or proceed to the file that is linked to it.

5.5 Sample Plate Filling Experiment

File 61: Plate Filling

The following example will show you how to perform a plate filling operation using a 24 across template. The plate will be in position 1.

Each well of the plate will be pre-filled with 25 µL of solution. There will be four mixes in each well after every transfer.

1. Fill an empty plate with 25 µL of diluent.
2. Place a "24 across" template on the table.
3. Place the magazine of tips in the back cavity of the template.
4. Place the plate in the front cavity of the template with samples.
5. Press "file", "61", and then "enter" to access File 612.
6. Edit the file using the following keystrokes:

Display Before Editing	Keystrokes	Display After Editing
PLATE FILLING STEP TO EDIT FILE #61	STEP	-
PLATE POSITION STEP	1	1
VOL IN WELL AT START (0-50) 25 ul	25; ENTER	VOL IN WELL AT START (0-50) 25 ul
DELIVER FROM T1 T2 T3	T1: ENTER	DELIVER FROM T1 T2 T3
MIXES IN MICROTROF 9 3 5	5, ENTER	MIXES IN MICROTROF
MICROTROF MIX VOLUME (0-200) 50ul	STEP (0-3)	MICROTROF MIX VOLUME 3 ul
DELIVERY VOLUME 0-200 50	DELIVERY VOLUME 75	0-200 75
MIXES AFTER TRANSFER	4	MIXES AFTER TRANSFER
MIX VOLUME 0-50 50	25	MIX VOLUME 25
PLATE TYPE FLAT-U-V-HEIGHT-TUBE	U	PLATE TYPE FLAT-U-V-HEIGHT-TUBE
FILL PLATE ROWS A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P	C	FILL PLATE ROWS A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P
MICROTROF VOL AT START (20-80) 77 mL	70	MICROTROF VOL AT START 20-80 70
CHANGE TIPS YES/NO YES	YES	CHANGE TIPS YES/NO YES
LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW		LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW
LINK TO STORED FILE (0-45, 0=STOP) 0	0	LINK TO STORED FILE (0-45, 0=STOP) 0
END OF FILE RUN-STORE		

6 SERIAL DILUTION

6.1 Overview

Serial Dilution is the progressive dilution and redilution of a liquid. In this process, a sample is added to a diluent and the two are mixed. An aliquot is then removed for addition to the next diluent-containing well. This sequence is repeated until the desired number of dilutions have been obtained ("serial dilutions"). As an example, if equal volumes of sample and diluent were mixed during each step of the series, the sample concentration would be 1:2, 1:4, 1:8, 1:16, 1:32, and so on in sequential rows.

The template for Serial Dilution is **File 62.** This file can be used as a template to establish a user file which can be stored and used as desired (and it can be as a template for additional serial dilution files).

6.2 Editing a Serial Dilution File

When you open File 62 (or any other Serial Dilution file), the opening step is presented and the step command should be selected to view the file on a screen-by-screen basis. You can change the value of each parameter as desired to provide the desired program to fill the plates. After a value is entered, press ENTER. To advance to the next step press STEP. A detailed discussion of role of each key is presented in Chapter 4.

1. OPENING STEP

SERIAL DILUTION
STEP TO EDIT FILE # 62

When File 62 is opened, the **OPENING STEP** display is presented. If you want to view/edit the file, press STEP.

2. VOL IN WELL AT START STEP

VOL IN WELL AT START	
(0-350)	20 ul

VOLUME IN WELL AT START is used to tell the system how much diluent is in each well before any transfer has been made. This parameter does not pertain to the first row, which contains the sample. The system uses this number to determine how far down into the wells the tips should go, and also to prevent the instrument from attempting to withdraw more sample than is actually present. This parameter can be set from 0 to 50 µL in 1 µL increments.

*****screen says 0-350 but text says 50

3. TRANSFER VOLUME

TRANSFER VOLUME	
(0-100)	5 ul

TRANSFER VOLUME indicates how much sample should be withdrawn from the original well for transfer to the new well during a serial dilution. This parameter can be set from 0 to 50 µL in 1 µL increments. A transfer volume of 0 may be used if you wish to simply mix the contents of the well.

4. **MIXES BEFORE TRANSFER**

MIXES BEFORE TRANSFER	
(0-9)	3

MIXES BEFORE TRANSFER is used to indicate the number of aspiration/delivery cycles that should be performed before transferring a sample. As an example, if you are diluting cells which may settle, the MIX BEFORE TRANSFER step might be used to agitate them back into suspension before transfer by mixing. This parameter can be set from 0 to 9 (0 indicates no mixing).

5. **MIX VOLUME**

MIX VOLUME	
(0-75)	5ul

MIX VOLUME is used to tell the system how much solution should be used in the Mix step. Typically this volume is set to 50% of the total volume in the well to be mixed, but will be more or less depending on the application (when mixing cells, it may be desirable to use a lower percentage of the total volume). The value of this parameter may not exceed 75% of the total volume in the well you are mixing. The system will automatically calculate this amount and indicate it on the display as the upper limit in the range. This parameter can be set in 1 µL increments from 0 µL to the upper limit.

6. **CHANGE TIPS**

CHANGE TIPS? YES/NO	YES

CHANGE TIPS is used to indicate if the tips should be removed after the first liquid is dispensed and a new set of tips from the tip magazine should be placed on the pipette head, thus decreasing carryover. If you don't want to change tips, then the same tips will be used throughout the entire operation.

7. **PLATE TYPE**

PLATE TYPE
FLAT-U-V-HEIGHT-TUBE

PLATE TYPE is used to indicate the design of the bottom of the plate. This information is used to determine the height of the liquid in each well and thus calculate how far down into the wells the tips should go. If you are not using a standard microplate, select HEIGHT and determine the amount to raise or lower the tip height by using File 70, the Tip Height File (Section x.xx). The height parameter can be set from 100 to 3500 in increments of 1.

8. **PLATE ORIENTATION**

PLATE ORIENTATION
24-16 WELLS ACROSS

PLATE ORIENTATION is used to indicate how the plate is positioned. The value that is selected indicates the number of wells that will be under the pipette tips at one time.

9. ROWS TO DILUTE

ROWS TO DILUTE
A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P

ROWS TO DILUTE is used to indicate the rows of the plate are to be included in the serial dilution. Rows are designated by letters A through P (back to front) in the "24 across" configuration and 1 through 16 in the "16 across" configuration. To select row A, press YES. The underline will remain and B will be active (if you do not want to use a row, press NO to advance to the next row). If you want all rows to be filled, press the STEP key. After the last NO selection has been made, press STEP (i.e. if you want all rows except for A, press NO for A. The active row will become B. If you press STEP, rows B-P will be active.

10. EMPTY LAST ROW TO

EMPTY LAST ROW TO
OFF-1ST ROW-MAG-MICROTROF3

EMPTY LAST ROW TO is used to tell the system where to place the extra volume of liquid transferred into the last row of wells after a serial dilution.

1. If you select OFF, the extra liquid will be left in the last row of wells.

2. If you select 1ST ROW, the extra liquid will be removed from the last row of wells and return it to the first row of wells you chose in ROWS TO DILUTE.

3. If you select MAG, the system will remove the extra liquid and discard it into the tip magazine.

4. If you select MICROTROF3, the system will remove the extra liquid and discard it into the rear reservoir of a MICROTROF container located in the P2 cavity of the template.

Note: Note: MICROTROF3 will appear on the display only when the 24 wells across PLATE ORIENTATION is selected.

11. LIQUID HANDLING SPEED

LIQ HANDLING SPEEDS
RAPID-MEDIUM-SLOW

LIQUID HANDLING SPEEDS is used to select the rate sample delivery from the tips (rapid, medium, or slow).

12. LINK TO STORED FILE

LINK TO STORED FILE	
(0-45, 0=STOP)	0

LINK TO STORED FILE is used to indicate what the system should do after it completes the file it is currently running.

If you want the system to stop at the end of the file, enter 0.

If you want the system to perform another file after the present file is complete, enter the desired file number. The LINK TO STORED FILE command permits more efficient operation because you don't need to start each individual file manually with the "file", "##" A detailed discussion of Linked files is presented in Section 10.

13. END OF FILE

END OF FILE
RUN-STORE

END OF FILE is used to indicate what the system should do after you have completed the editing of the file.

If you select RUN, the file will start and step 14 will be presented.

If you select STORE, the file will be copied into memory (see Section 6.3 for additional information about storing files).

14. ENTER FIRST TIP ROW

ENTER FIRST TIP ROW	
(A-P)	P

ENTER FIRST TIP ROW is used to specify which row of tips to use. Rows of the tip magazine are designated by letters A through P (back to front).

6.3 Storing/Overwriting the File

To store a new file:

1. At the END OF FILE statement (item p, Section 5.2), select STORE. The display will appear as shown below.

FILE STORE
ENTER USER # _____

2. Enter your user number or project number (this number is provided for reference only and does not affect the use of the system), then press ENTER. The display will indicate:

FILE STORE
ENTER FILE #XX

   Where xx is the file number assigned by the system, which will be the lowest unoccupied file. If you want to assign a different number, enter it.

3. Press ENTER. The display will present:

YES TO STORE
USER #zzzz FILE #XX

4. Press YES to accept the assignment.

6.4 Running a Serial Dilution File

When you are ready to run a Serial Dilution:

1. Select the desired file.

2. Press **START.**

If the MICROTROF does not contain the required amount of liquid to perform the desired operation, the message **PLEASE FILL MICROTROF** will appear on the screen. This prompt allows you to simply enter the volume you are adding to the MICROTROF reservoir without requiring you to step back through each of the file's parameters.

At the conclusion of the operation, the system will stop or proceed to the file that is linked to it.

6.5 Sample Serial Dilution Experiment

File 62: Serial Dilution: 1:2 Ratio

The following example will show you how to perform a simple 1:2, 1:4, 1:8, 1:16, etc. serial dilution on 5 µL samples in an "16 across" microplate. Each well of the plate will be pre-filled with 25 µL of solution. There will be four mixes in each well after every transfer.

1. Fill an empty plate with 25 µL of diluent.
2. Place a "16 across" template on the table.
3. Place the magazine of tips in the back cavity of the template.
4. Manually pipette 50 µL samples into row 1 of the plate. Use water in this example.
5. Place the plate in the front cavity of the template with samples.
6. Press "file", "62", and then "enter" to access File 62.
7. Edit the file using the following keystrokes:

Display Before Editing	Keystrokes	Display After Editing
SERIAL DILUTION STEP TO EDIT FILE #62	STEP	-
VOL IN WELL AT START (0-50) 25 ul	25; ENTER	VOL IN WELL AT START (0-50) 25 ul
TRANSFER VOLUME (0-50) 5 ul	STEP	TRANSFER VOLUME (0-50) 5 ul
MIXES BEFORE TRANSFER (0-9) 3	STEP	MIXES BEFORE TRANSFER (0-9) 3
MIX AFTER TRANSFER (0-9) 3	STEP	MIX AFTER TRANSFER (0-9) 3
MIX VOLUME (0-3) 3 ul	STEP	MIX VOLUME (0-3) 3 ul
CHANGE TIPS? YES-NO	STEP	CHANGE TIPS? YES-NO
PLATE TYPE FLAT-U-V-HEIGHT-TUBE	NO; STEP	PLATE TYPE FLAT-U-V-HEIGHT-TUBE
PLATE ORIENTATION 24-16 WELLS ACROSS	NO; STEP	PLATE ORIENTATION 24-16 WELLS ACROSS
DILUTE ROWS 1-2-3- 4-5-6-7-8-9-10-11-12 13-14-15-16-17-18-19 -20-21-22-23-24	STEP	DILUTE ROWS 1-2-3- 4-5-6-7-8-9-10-11-12- 13-14-15-16-17-18-19 20-21-22-23-24
EMPTY LAST ROW TO OFF-1ST ROW-MAG-TROF3	STEP	EMPTY LAST ROW TO OFF-1ST ROW-MAG-TROF3
LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW	STEP	LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW
LINK TO STORED FILE (0-45, 0=STOP) 0	STEP	LINK TO STORED FILE (0-45, 0=STOP) 0
END OF FILE RUN-STORE	ENTER	-
SERIAL DILUTION START TO RUN FILE #62	START	-

Serial dilutions can be very cumbersome and time consuming when performed manually. The WellPro liquid handler has the capability to perform very complex serial dilution sequences. As you gain familiarity with the WellPro liquid handler and especially the powerful **FILE LINKING** features, experiment with the range of possible sequences to customize your own complex serial dilution files.

7 PLATE TO PLATE TRANSFER

7.1 Overview

Transferring an aliquot of sample from one plate to another is called "plate-to-plate" transfer or "replica plating." This technique allows for transfer of samples in a SOURCE plate to a RECEIVER plate for assay, culture, etc.

The steps required to perform the plate transfer are:

1. Aspirate sample from source plate.

2. Deliver sample to receiver plate.

3. Replace used tip with fresh one and proceed to next sample row, etc.

The template for Plate to Plate Transfer is File 63, which is used as a template to establish a user file which can be stored and used as desired (and it can be as a template for additional serial dilution files).

7.2 Types of Plate to Plate Transfer

There are two general types of Plate to Plate Transfer:

1. transfer any single row in one plate to any other single row in another plate.

2. transfer any single row in one plate to two or more rows in another plate.

The program allows you to specify the direction of the transfer. The default direction is from plate 2 to plate 1, but the system also allows you to transfer from plate 1 to plate 2, so that either plate can be used as the DESTINATION plate. As examples, you can transfer from row A of plate 1 to row B of plate 2 or you can transfer from row A of plate 2 to rows A, B, and C of plate 1. This enables you to set up an assay plate to do duplicates, triplicates, etc.

The **TRANSFER MODE/DIRECTION** is a feature that is especially useful when it is combined with **FILE LINK** because it allows you to transfer duplicate samples from the rows of one plate to another plate. Thus, using these functions in combination makes it possible to transfer from row A of plate 1 to rows A and B of plate 2, then automatically continue with another file which transfers from row B of plate 1 to rows C and D of plate 2, and so on.

TRANSFER MODE/DIRECTION also allows you to use a 384 well plate as a reagent MICROTROF to deliver multiple reagents to a single assay plate. You can place the same or different reagents in each of the 24 columns, and/or each of the 16 rows of the source plate. These reagents can be delivered to specified rows on the destination plate. This arrangement minimizes reagent waste.

When you use TRANSFER MODE/DIRECTION feature with the TUBE HANDLING feature option, you can carry out transfers from larger containers such as micro test tubes or segmented troughs and use them as reagent containers. Using the "help" key enables you to extend the acceptable range for TRANSFER VOLUME and VOLUME IN WELL AT START, so you can make full use of the larger volume capacity of these containers

7.3 Editing a Plate to Plate Transfer File

When you open file 63 (or any other Serial Dilution file), the opening step is presented and the step command should be selected to view the file on a screen-by-screen basis. You can change the value of each parameter as desired to provide the desired program to fill the plates. After a value is entered, press ENTER. To advance to the next step press STEP. A detailed discussion of role of each key is presented in Chapter 4.

1. OPENING STEP

PLATE TO PLATE
STEP TO EDIT FILE #63

When File 63 is opened, the **OPENING STEP** display is presented. If you want to view/edit the file, press STEP.

2. TRANSFER DIRECTION

TRANSFER DIRECTION
P2 to P1 - P1 to P2

TRANSFER DIRECTION is used to indicate whether the system should transfer from plate 2 to plate 1 or from plate 1 to plate 2 (i.e. either plate 1 (the plate in the front cavity of the template) or plate 2 (the plate in the middle cavity) can be used as the source plate.

3. VOL IN WELL PLATE 1

VOL IN WELL PLATE 1	
(0-50)	0 ul

VOLUME IN WELL PLATE 1 is used to indicate how much liquid is in each well of plate 1. The system uses this value to determine how far down into the wells the tips should go and also to prevent exceeding the volume capacity of the wells. This parameter can be set from 0 to 50 µL in 1 µL increments.

4. VOL IN WELL PLATE 2

VOL IN WELL PLATE 2	
(0-50)	50 ul

VOLUME IN WELL PLATE 2 is used to indicate how much liquid is in each well of plate 2. The system uses this value to determine how far down into the wells the tips should go and also to prevent exceeding the volume capacity of the wells. This parameter can be set from 0 to 50 µL in 1 µL increments.

5. TRANSFER MODE

TRANSFER MODE
1 TO 1-1 TO SEVERAL

TRANSFER MODE is used to tell the system whether to transfer one row of plate 1 to the corresponding row of plate 2, or to transfer one row of plate 1 to several different or multiple rows of plate 2.

6. **TRANSFER VOLUME**

TRANSFER VOLUME	
(0-50)	25 ul

TRANSFER VOLUME is used to tell the system how much sample to withdraw from a source well for transfer to a new well during a plate transfer. This parameter can be set from 0 to 50 µL in 1 µL increments. The system calculates the maximum TRANSFER VOLUME for the row by dividing the VOLUME IN WELL AT START by the number of transfers specified for that row.

7. **MIXES BEFORE TRANSFER**

MIXES BEFORE TRANSFER	
(0-9)	0

MIXES BEFORE TRANSFER is used to indicate the number of aspiration/delivery cycles that should be performed before transferring a sample. For example, when you are diluting cells which may settle, you should agitate them back into suspension before transfer by mixing. This parameter can be set from 0 to 9 (0 indicates no mixing).

8. **MIXES AFTER TRANSFER**

MIXES AFTER TRANSFER	
(0-9)	0

MIXES AFTER TRANSFER is used to indicate the number of aspiration/delivery cycles that should be performed after transferring a sample. For example, when you are diluting cells which may settle, you should agitate them back into suspension after transfer by mixing. This parameter can be set from 0 to 9 (0 indicates no mixing).

9. **MIX VOLUME**

MIX VOLUME	
(0-25)	25 ul

MIX VOLUME is used to tell the system how much solution should be used in the Mix step. Typically this volume is set to 50% of the total volume in the well to be mixed, but will be more or less depending on the application. For example, when mixing cells it may be desirable to use a lower percentage of the total volume. The value of this parameter may not exceed 75% of the total volume in the well you are mixing. The system will automatically calculate this amount and indicate it on the display as the upper limit in the range. This parameter can be set in 1 µL increments from 0 µL to the upper limit.

10. **CHANGE TIPS**

CHANGE TIPS?
YES-NO

CHANGE TIPS is used to indicate if the tips should be removed after the first liquid is dispensed and a new set of tips from the tip magazine should be placed on the pipette head, thus decreasing carryover. If you don't want to change tips, then the same tips will be used throughout the entire operation.

11. PLATE 1 TYPE

PLATE 1 TYPE
FLAT-U-V-HEIGHT-TUBE

PLATE 1 TYPE is used to indicate the design of the bottom of plate 1. This information is used to determine the height of the liquid in each well and thus calculate how far down into the wells the tips should go. If you are not using a standard microplate, select HEIGHT and determine the amount to raise or lower the tip height by using File 70, the Tip Height File (Section x.xx). The height parameter can be set from 100 to 3500 in increments of 1.

12. PLATE 2 TYPE

PLATE 2 TYPE
FLAT-U-V-HEIGHT-TUBE

PLATE 2 TYPE is used to indicate the design of the bottom of plate 2. This information is used to determine the height of the liquid in each well and thus calculate how far down into the wells the tips should go. If you are not using a standard microplate, select HEIGHT and determine the amount to raise or lower the tip height by using File 70, the Tip Height File (Section x.xx). The height parameter can be set from 100 to 3500 in increments of 1.

13. SOURCE ROW

SOURCE ROW
A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P

SOURCE ROW is used to indicate which row of the SOURCE plate is used to withdraw samples from. This parameter only appears if the "1 to SEVERAL" mode is selected in this file. In that instance, the SOURCE row specified acts as the single source row for transfer to several receiving or DESTINATION rows.

14. TRANSFER ROW

TRANSFER ROW
A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P

TRANSFER ROW is used to indicate which row of the TRANSFER plate is used to place liquid in during transfer. This parameter only appears if the "1 to SEVERAL" mode is selected in this file. In that instance, the SOURCE row specified acts as the single source row for transfer to several receiving or DESTINATION rows.

15. DESTINATION ROWS

DESTINATION ROWS
A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P

DESTINATION ROW is used to indicate which row of the DESTINATION plate is used to place liquid in during transfer. This parameter only appears if the "1 to SEVERAL" mode is selected in this file. In that instance, the SOURCE row specified acts as the single source row for transfer to several receiving or DESTINATION rows.

16. LIQ HANDLING SPEEDS

LIQ HANDLING SPEEDS
RAPID-MEDIUM-SLOW

LIQ HANDLING SPEEDS is used to select the rate of sample delivery from the tips (rapid, medium, or slow).

17. LINK TO STORED FILE

LINK TO STORED FILE	
(0-45, 0=STOP)	0

LINK TO STORED FILE is used to indicate what the system should do after it completes the file it is currently running.

If you want the system to stop at the end of the file, enter 0.

If you want the system to perform another file after the present file is complete, enter the desired file number. The LINK TO STORED FILE command permits more efficient operation because you don't need to start each individual file manually with the "file", "##" A detailed discussion of Linked files is presented in Section 10.

18. END OF FILE

END OF FILE
RUN-STORE

END OF FILE is used to indicate what the system should do after you have completed the editing of the file:

If you select RUN, the file will start.

If you select STORE, the file will be copied into memory (see Section 6.3 for additional information about storing files).

19. ENTER FIRST TIP ROW

ENTER FIRST TIP ROW	
(A-P)	P

ENTER FIRST TIP ROW is used to specify which row of tips to use. Rows of the tip magazine are designated by letters A through P (back to front).

7.4 Storing/Overwriting the File

To store a new file:

1. At the END OF FILE statement (item p, Section 5.2), select STORE. The display will appear as shown below.

FILE STORE
ENTER USER # _____

2. Enter your user number or project number (this number is provided for reference only and does not affect the use of the system, then press ENTER. The display will indicate:

FILE STORE
ENTER FILE #XX

   Where xx is the file number assigned by the system, which will be the lowest unoccupied file. If you want to assign a different number, enter it.

3. Press ENTER. The display will present:

YES TO STORE
USER #zzzz FILE #XX

4. Press YES to accept the assignment.

7.5 Running a Plate to Plate Transfer

When you are ready to run a plate to plate transfer:

1. Select the desired file.

2. Press **START.**

If the MICROTROF does not contain the required amount of liquid to perform the desired operation, the message **PLEASE FILL MICROTROF** will appear on the screen. This prompt allows you to simply enter the volume you are adding to the MICROTROF reservoir without requiring you to step back through each of the file's parameters.

At the conclusion of the operation, the system will stop or proceed to the file that is linked to it.

7.6 Sample Serial Dilution Experiments

File 63: Plate to Plate Transfer

The following example will show you how to perform a simple transfer of 30 µL of a simulated cell suspension from plate 2 to plate 1 which contains no liquid in its wells prior to transfer. There will be four 30 µL medium-speed mixes in each well before solution is transferred, as might be the case when you want to gently agitate cells into suspension before transferring them. You will be using flat-bottomed plates.

1. Place a "24 across" template on the table.

2. Place a magazine of tips in the back cavity of the template.

3. For demonstration purposes, manually pipette 50 µL of water into each of the 384 wells of plate #2.

4. Place the plate in the middle cavity of the template.

5. Place an empty plate in the front cavity of the template.

6. Press "file", "63", and then "enter" to access File 63.

7. Edit the file using the following keystrokes:

Display Before Editing	Keystrokes	Display After Editing
PLATE-TO-PLATE STEP TO EDIT FILE #63	STEP	-
TRANSFER DIRECTION P2 TO P1 - P1 TO P2	STEP	TRANSFER DIRECTION P2 TO P1 - P1 TO P2
VOL IN WELL PLATE 1 (0-50) 0 ul	STEP	VOL IN WELL PLATE 1 (0-50) 0 ul
VOL IN WELL PLATE 2 (0-50) 50 ul	STEP	VOL IN WELL PLATE 2 (0-50) 50 ul
TRANSFER MODE 1 TO 1-TO SEVERAL	STEP	TRANSFER MODE 1 TO 1-TO SEVERAL
TRANSFER VOLUME (0-50) 30ul	70; ENTER	TRANSFER VOLUME (0-50) 30ul
MIXES BEFORE TRANSFER (0-9) 0	4; ENTER	MIXES BEFORE TRANSFER (0-9) 4
MIXES AFTER TRANSFER (0-9) 0	STEP	MIXES AFTER TRANSFER (0-9) 0
MIX VOLUME (0-22) 22 ul	22; ENTER	MIX VOLUME (0-22 ul) 22 ul
CHANGE TIPS? YES-NO	STEP	CHANGE TIPS? YES-NO
PLATE 1 TYPE FLAT-U-V-HEIGHT-TUBE	STEP	PLATE 1 TYPE FLAT-U-V-HEIGHT-TUBE
PLATE 2 TYPE FLAT-U-V-HEIGHT-TUBE	STEP	PLATE 2 TYPE FLAT-U-V-HEIGHT-TUBE
TRANSFER ROWS A-B-C-D-E-F-G-H I-J-K-L-M-N-O-P	STEP	TRANSFER ROWS A-B-C-D-E-F-G-H I-J-K-L-M-N-O-P
LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW	NO; STEP	LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW
LINK TO STORED FILE (0-45, 0-STOP) 0	STEP	LINK TO STORED FILE (0-45, 0-STOP) 0
END OF FILE RUN-STORE	ENTER	-
PLATE TO PLATE START TO RUN FILE #63	START	-

File 63: Plate to Plate Transfer Mode and Direction

The following example will show you how to use the TRANSFER MODE capability in combination with File 63: Plate-to-Plate Transfer to transfer 35 µL of water, simulating sample. The WellPro liquid handler allows you to specify the direction of the transfer. You can designate either plate as the **SOURCE** or the **DESTINATION** plate.

In this case the transfer will be from row B of plate 1 to rows C and D of plate 2.

1. Place a "24 across" template on the table.
2. Place a magazine of tips in the back cavity of the template.
3. Place the source plate into the front cavity of the template. This will be the source plate.
4. Place an empty plate in the middle cavity of the template. This will be the destination plate, plate #2.
5. Press "file", "63", and then "enter" to access File 63.
6. Edit the file using the following keystrokes:

Display Before Editing	Keystrokes	Display After Editing
PLATE TO PLATE STEP TO EDIT FILE #63	STEP	-
TRANSFER DIRECTION P2 TO P1 - P1 TO P2	NO;STEP	TRANSFER DIRECTION P2 TO P1 - P1 TO P2
VOL IN WELL PLATE 1 (0-50) 50 ul	STEP	VOL IN WELL PLATE 1 (0-50) 50 ul
VOL IN WELL PLATE 2 (0-50) 0 ul	STEP	VOL IN WELL PLATE 2 (0-50) 0 ul
TRANSFER MODE 1 TO 1-1 TO SEVERAL	STEP	TRANSFER MODE 1 TO 1- 1 TO SEVERAL
SOURCE ROW A-B-C-D-E-F-G-H	NO; STEP	SOURCE ROW A-B-C-D-E-F-G-H
DESTINATION ROWS A-B-C-D-E-F-G-H	NO; NO; YES; YES; NO; NO; NO; NO; STEP	DESTINATION ROWS --C-D- - - -

Note: In the next step, the WellPro controller automatically calculates the acceptable range of the TRANSFER VOLUME by dividing the VOL IN WELL PLATE 1 (50 µl) by the number of rows a transfer will be made to (two rows, C and D). The maximum acceptable volume for the transfer is therefore 25 µl.

Display Before Editing	Keystrokes	Display After Editing
TRANSFER VOLUME (0-50) 50 ul	25; ENTER	TRANSFER VOLUME (0-50) 25 ul
MIXES BEFORE TRANSFER (0-9) 0	STEP	MIXES BEFORE TRANSFER (0-9) 0
MIXES AFTER TRANSFER (0-9) 0	STEP	MIXES AFTER TRANSFER (0-9) 0
CHANGE TIPS? YES-NO	STEP	CHANGE TIPS? YES-NO
PLATE 1 TYPE FLAT-U-V-HEIGHT-TUBE	STEP	PLATE 1 TYPE FLAT-U-V-HEIGHT-TUBE
PLATE 2 TYPE FLAT-U-V-HEIGHT-TUBE	STEP	PLATE 2 TYPE FLAT-U-V-HEIGHT-TUBE
LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW	STEP	LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW
LINK TO STORED FILE (0-45, 0=STOP) 0	STEP	LINK TO STORED FILE (0-45, 0=STOP) 0
END OF FILE RUN-STORE	ENTER	-
PLATE TO PLATE START TO RUN FILE #63	START	-

Note: The WellPro liquid handler allows you to create and store multiple versions of File 63: Plate-to-Plate Transfer, so as to transfer from row A of plate 1 to rows A and B of plate 2, from row B to rows C and D, from row C to rows E and F, from row D to rows G and H, and then link these files together. The result is that you can *conveniently transfer from the rows of one plate to duplicate rows of another plate for assay*, using a single "start" keystroke. Details on file linking are provided in Section 10.

8 EMPTYING A PLATE

8.1 Overview

The WellPro liquid handler can remove all or part of the solution in a plate and replace it with another solution which is delivered from a MICROTROF reservoir.

The steps that the WellPro liquid handler uses to empty a plate.are:

1. Aspirate sample from plate.

2. Deliver to "T3" waste reservoir.

The template for Emptying a plate is File 64, which is used as a template to establish a user file that can be stored and used as desired (and it can be used as a template for additional **Emptying a plate** files

Note: This file is designed to make rapid exchanges of culture media, reagents, diluents, etc. within a single plate. For refilling with a new reagent, link to an edited FILE # 61, plate fill file.

8.2 Editing an Emptying a Plate File

When you open file 64 (or any other Serial Dilution file) the opening step is presented and the step command should be selected to view the file on a screen-by-screen basis. You can change the value of each parameter as desired to provide the desired program to fill the plates. After a value is entered, press ENTER. To advance to the next step press STEP. A detailed discussion of role of each key is presented in Chapter 4.

1. OPENING STEP

PLATE EMPTY
STEP TO EDIT FILE # 64

When File 64 is opened, the **OPENING STEP** display is presented. If you want to view/edit the file, press STEP.

2. VOL IN WELL AT START

VOL IN WELL AT START	
(0-50)	50 ul

VOLUME IN WELL AT START is used to how much liquid is in each well before any solution has been removed. The system uses this number to determine how far down into the wells the tips should go and also to prevent the instrument from attempting to withdraw "present". This parameter can be set from 0 to 50 µL in 1 µL increments.

3. VOLUME TO EMPTY

VOLUME TO EMPTY	
(0-50)	50 ul

VOLUME TO EMPTY: is used to indicate how much liquid to remove from each well and transfer to the waste container, a reservoir or the MICROTROF container. This parameter can be set from 0 to 50 µL in 1 µL increments.

4. WASTE TO MICROTROF

WASTE TO MICROTROF
T1-T2-T3

WASTE TO MICROTROF is used to indicate which reservoir of the MICROTROF container to deliver its waste solution to. This parameter can be set to T1, T2, or T3, which indicate respectively the front, middle, and rear reservoirs.

5. CHANGE TIPS

CHANGE TIPS
YES-NO

CHANGE TIPS is used to indicate whether you want it to get new tips from the tip magazine during the waste removal, thus decreasing carryover. If you don't want to change tips, the system will use the same tips for the whole plate.

6. PLATE TYPE

PLATE TYPE
FLAT-U-V-HEIGHT-TUBE

PLATE TYPE is used to indicate the design of the bottom of the plate. This information is used to determine the height of the liquid in each well and thus calculate how far down into the wells the tips should go. If you are not using a standard microplate, select HEIGHT and determine the amount to raise or lower the tip height by using File 70, the Tip Height File (Section 10). The height parameter can be set " from 100 to 3500 in increments of 1.

7. MIXES BEFORE EMPTY

MIXES BEFORE EMPTY	
(0-9)	0

MIXES BEFORE EMPTY is used to tell the system how many mixes (aspiration/delivery cycles) should be performed during the mixing process before waste removal. You can set the value of this parameter from 0 to 9 mixes in increments of 1. The numeral 0 indicates no mixing

8. ROWS TO EMPTY

ROWS TO EMPTY
A-B-C-D-E-F-G-H-I-J-K-L-M-N-O-P

ROWS TO EMPTY is used to indicate the rows of the plate to empty and which to leave illed. Rows are designated by letters A through P. To select row A, press YES. The underline will remain and B will be active (if you do not want to use a row, press NO to advance to the next row). If you want all rows to be emptied, press the STEP key. After the last NO selection has been made, press STEP (i.e. if you want all rows except for A, press NO for A. The active row will become B. If you press STEP, rows B-P will be active.

9. LIQUID HANDLING SPEEDS

LIQ HANDLING SPEEDS
RAPID-MEDIUM-SLOW

LIQUID HANDLING SPEEDS is used to select the rate sample withdrawal by the tips (rapid, medium, or slow).

10. LINK TO STORED FILE

LINK TO STORED FILE	
(0-45, 0=STOP)	0

LINK TO STORED FILE is used to indicate what the system should do after it completes the file it is currently running.

If you want the system to stop at the end of the file, enter 0.

If you want the system to perform another file after the present file is complete, enter the desired file number. The LINK TO STORED FILE command permits more efficient operation because you don't need to start each individual file manually with the "file", "##" A detailed discussion of Linked files is presented in Section 10.

11. END OF FILE

END OF FILE
RUN-STORE

END OF FILE is used to indicate what the system should do after you have completed the editing of the file.

If you select RUN, the file will start.

If you select STORE, the file will be copied into memory (see Section 5.3 for additional information about storing files).

Note: You can also review the file you have just made by continuing to press the STEP key.

12. ENTER FIRST TIP ROW

ENTER FIRST TIP ROW
(A-P) P

ENTER FIRST TIP ROW is used to specify which row of tips to start with. Rows of the tip magazine are designated by letters A through P (from back to front).

8.3 Storing/Overwriting the File

To store a new file:

1. At the **END OF FILE** statement (item 11, Section 8.2), select STORE. The display will appear as shown below.

FILE STORE
ENTER USER # _____

2. Enter your user number or project number (this number is provided for reference only and does not affect the use of the system, then press ENTER. The display will indicate:

FILE STORE
ENTER FILE #XX

   Where xx is the file number assigned by the system, which will be the lowest unoccupied file. If you want to assign a different number, enter it.

3. Press ENTER. The display will present:

YES TO STORE
USER #zzzz FILE #XX

4. Press YES to accept the assignment.

8.4 Running an Empty a Plate File

When you are ready to run an Empty a Plate file

1. Select the desired file.

2. Press **START.**

At the conclusion of the operation, the system will stop or proceed to the file that is linked to it.

9 TIME DELAY FILE

9.1 Overview

A Time Delay file allows you to insert a delay between two files that have been linked into a sequence. This time delay can be specified from 1 second in length up to several hours. Extremely long time delays are possible by linking two or more delay files in sequence. This lets you more fully automated the liquid handling portions of assays.

As an example of the utility of this feature, you can direct the system to add a reagent, wait a specified period of time and then add another reagent. You can also use a plate-to-plate transfer to start a reaction, pause to incubate and then use a plate fill operation to stop the reaction.

9.2 Editing a Time Delay File

When you open file 65 (or any other Time Delay file) the opening step is presented and the step command should be selected to view the file on a screen-by-screen basis. You can change the value of each parameter as desired to provide the desired program to fill the plates. After a value is entered, press ENTER. To advance to the next step press STEP. A detailed discussion of role of each key is presented in Chapter 4.

1. **OPENING STEP**

TIME DELAY
STEP TO EDIT FILE #65

When File 65 is opened, the **OPENING STEP** display is presented. If you want to view/edit the file, press STEP.

2. **DELAY**

DELAY
0 MINS 0 SEC

DELAY is used to indicate to the system to wait a specified interval after completing a file in a linked sequence before beginning the next file in that sequence. You can set the length of the delay from 0 to 999:59 (minutes:seconds. When the DELAY prompt first appears, the underline will allow you to enter the minutes field. When you press the Enter key, the seconds field will be activated.

****in the orig. that I have, the delay parameter only is 0-999 and 0-99

DELAY
(0-99) 0 MIN 0 SEC

3. PAUSE WHEN LINKING

PAUSE WHEN LINKING
NO-YES

PAUSE WHEN LINKING is used to tell the system whether to pause and await re-start after operator attention or whether it should continue on to the next file without halting. This feature is useful for changing plates or tips, adding reagents, etc.

4. LINK TO STORED FILE

LINK TO STORED FILE
(0-45, 0 = STOP) 0

LINK TO STORED FILE is used to indicate what the system should do after it completes the file it is currently running.

If you want the system to stop at the end of the file, enter 0.

If you want the system to perform another file after the present file is complete, enter the desired file number. The LINK TO STORED FILE command permits more efficient operation because you don't need to start each individual file manually with the "file", "##" A detailed discussion of Linked files is presented in Section 10.

5. END OF FILE

END OF FILE
RUN-STORE

END OF FILE is used to indicate what the system should do after you have completed the editing of the file:

- If you select RUN, the file will start.
- If you select STORE, the file will be copied into memory (see Section 5.3 for additional information about storing files).

Note: You can also review the file you have just made by continuing to press the STEP key.

9.3 Storing/Overwriting the File

To store a new file:

1. At the END OF FILE statement (item 5, Section 8.2), select STORE. The display will appear as shown below.

FILE STORE
ENTER USER # _____

2. Enter your user number or project number (this number is provided for reference only and does not affect the use of the system, then press ENTER. The display will indicate:

FILE STORE
ENTER FILE #XX

   Where xx is the file number assigned by the system, which will be the lowest unoccupied file. If you want to assign a different number, enter it.

3. Press ENTER. The display will present:

YES TO STORE
USER #zzzz FILE #XX

4. Press YES to accept the assignment.

9.4 Using a Time Delay File

A time delay file is used with linked files. A detailed discussion of linked files is presented in Section 10.

While a Time Delay file is active, the display will show the time remaining.

10 LINKING FILES

10.1 Overview

The FILE LINK feature is provided to allow you to join two (or more) stored files as a sequence enabling the user to automate very complex liquid-handling operations. A file parameter, LINK TO STORED FILE, appears just before the END OF FILE in each file type (e.g. see Chapter 7). This prompt permits you to direct the WellPro liquid handler to transfer control to another file immediately after completing the file that it is currently running instead of terminating the operation of the file.

10.2 Using Linked Files

When the system is operating using a linked file sequence, the sequence will use the tips indicated in the ENTER FIRST TIP ROW prompt for the first file in the sequence. This prompt appears regardless of whether the tips are to be changed in the first file. The user should respond by specifying the row of tips (A-P, the standard magazine plus the tips for the MICROTROF™ container) that you want the WellPro liquid handler to select first.

The WellPro uses the same "counter" to keep track of which tips have been used, whether you have specified tip changes or not. Accordingly, each time the WellPro liquid handler links to the next file in a chain, it sequences through the tip magazine to select the next row of fresh, uncontaminated tips. Likewise, if you start a new file, or restart the same file, the counter automatically increments, causing the WellPro liquid handler to select the next row of unused tips.

When the last row of tips (row A) has been used, the WellPro liquid handler stops automatically and displays the ENTER FIRST TIP ROW prompt again. Change the magazine and specify the row of tips that you want to start with (usually row H for the magazine, or row J if you have also changed the tips for the MICROTROF container).

10.3 A Typical Series of Operation Using Linked Files

This section describes the use a linked file in a typical scenario.

In this example:

1. A sample will be diluted (File 21).

2. A small fraction of the diluted sample will be transferred to a new plate (File 22).

3. A reagent is added to each well (File 34).

File 21 is a user-customized Serial Dilution file. The last line in the file indicates that the operation should be transferred to file 22 at the termination of File 21.

LINK TO STORED FILE
(0-45, 0=STOP) 22

File 22 is a plate-to-plate transfer, in which a small sample from the diluted plate (from File 21) is pipetted to a new assay plate. The last line in the file indicates that the operation should be transferred to File 34 at the termination of File 22.

LINK TO STORED FILE (0-45, 0=STOP) 34

After the WellPro liquid handler has finished File 22, the operation of the system is transferred to File 34, which is used to add reagent via the manifold system. This is the final step in the overall operation as indicated by:

LINK TO STORED FILE (0-45, 0=STOP) 0

Note: The number of the files that are linked can be in any order (i.e. the file number is merely used for identification purposes.

11 ADJUSTING THE TIP HEIGHT

11.1 Overview

The depth of the tips in the wells must be properly set in order for the system to accurately aspirate fluid. When standard microplates are used, this depth is set by choosing one of the preset values – "FLAT", "U", or "V" that is presented in the PLATE TYPE parameter in the various files. However, when other plates are used (in a few special situations), it will be necessary to manually set the tip height using File 70.

The figures below illustrate the height positions that this file provides:

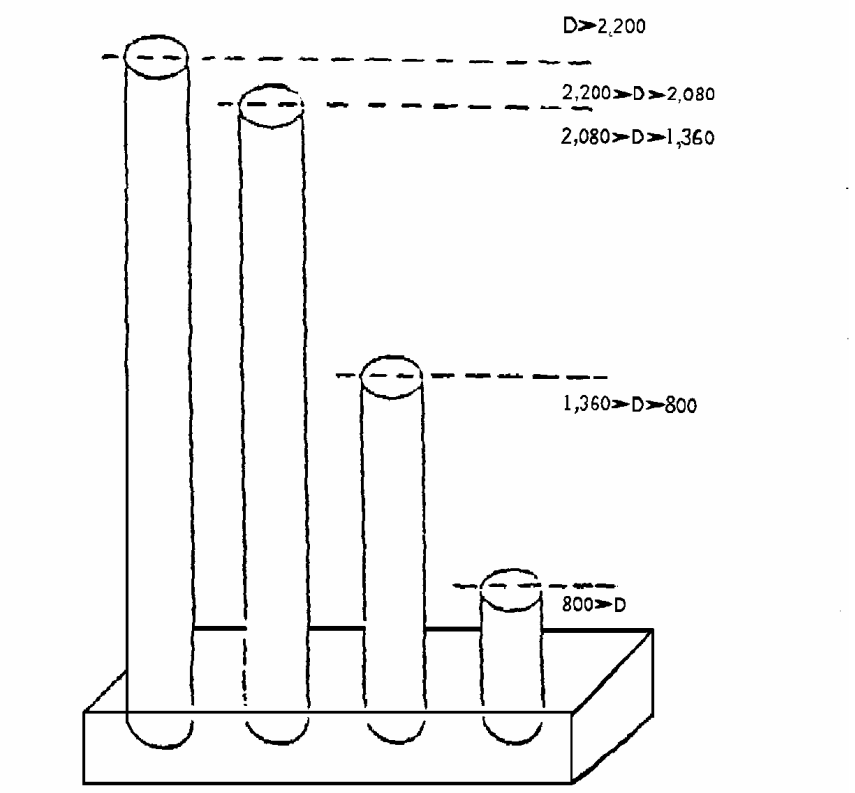

Fig. 11.1 Important "Height" Values

11.1.1 Editing/Running the Tip Height Adjustment File

When you open file 70 (the opening step is presented and the Start command should be selected to view the file on a screen-by-screen basis. You can change the value of each parameter as desired to provide the desired program to fill the plates. After a value is entered, press ENTER. To advance to the next step press STEP. A detailed discussion of role of each key is presented in Chapter 4.

1. **TIP HEIGHT**

TIP HEIGHT
START TO RUN FILE #70

Press **Start** to initiate the file

2. **SELECT FUNCTION**

SELECT FUNCTION
TIPS - WASH HEAD

Select Function is used to select measuring plate dimensions for use with the disposable tips.

3. **TIP NOW AT xxxx**

TIP NOW AT xxxx
(20 - 3500) GO TO ___

TIP NOW AT is used to raise (lower) the tips so that the tip is in a position that approximates the position you are trying to determine. You can set this parameter is desired from 20 to 3500 in increments of 1. Each step is equivalent to lowering the tip by approximately 0.00124 inch. As an alternative, you can use the "↑" OR "↓" keys to either raise or lower the tips ten steps at a time (0.0124 inch) in order to "fine adjust" the position of the tips.

When the tip is properly adjusted, press Enter to present the Tip Height screen.

4. **TIP HEIGHT**

TIP HEIGHT
COMPLETE FILE #70

11.1.2 Setting the Tip Height

Lower the tips until they are level with the top of the tubes. Record the TIP NOW AT number on a worksheet as the TUBE HEIGHT.

Note: Note the maximum number in the acceptable range at the lower left of the display. Subtract the TIP NOW AT number from the maximum that is indicated. The resulting value is very important. This difference allows you to determine which operations you can safely carry out with the tubes in question.

Figure 10.2 defines the operations you can and cannot carry out for differences in particular ranges. D is the difference between two values: The height of tube as it stands in the rack, and the maximum number shown in the range for the TIP NOW AT prompt of File 70: Tip Height File.

Fig 11.2. Tube Heights

Using Tall or Narrow Tubes

If your tubes are very tall and narrow, it is possible that the tips may not be able to reach the bottom of the tubes without forming a seal with the tube, or the "fins" on their upper edges may strike the top of the tubes. In the same vein, if the bottom of the tube is a sharply angled "V", the tips may get stuck in the tubes.

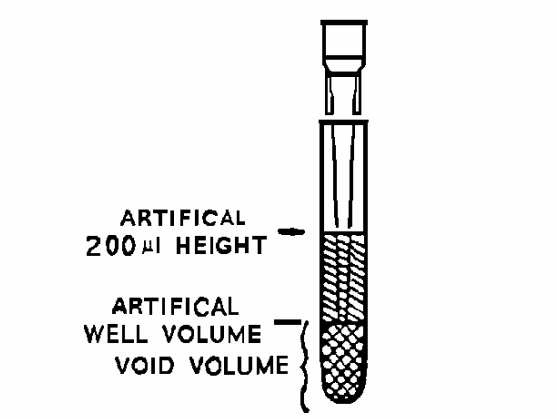

Fig.11 3 Tall and Narrow Tubes

If any of these problems are observed, it will be necessary to establish an artificial WELL BOTTOM HEIGHT above this point as described below:

1. Take a tube in one hand, and with the other hand lower a tip into the tube just far enough so that the "fins" don't touch the top of the tube, and the tip doesn't seal with the sides of the tube.

2. Mark the tube to show the position o the bottom of the tip. This will represent the artificial bottom. Manually pipette water into the tube until the meniscus reaches the mark you have made. This volume of water represents the "void volume". Record the value for "void volume" on the worksheet.

3. Place the tube into a rack and insert the rack in the middle cavity.

4. Use File 70 to determine the height of the water in the tube and record the TIP NOW AT number on a worksheet as the value for WELL BOTTOM HEIGHT.

5. Pipet 50 µl of water into the tube. Determine the height of liquid in the tube and record the TIP NOW AT number on a worksheet as the 50 UL HEIGHT.

Note: When using tall or narrow tubes, respond to the prompts on the display by entering the values you have just determined for WELL BOTTOM HEIGHT and 50 UL HEIGHT. Before carrying out any liquid-handling operations that involve tips, you must add an extra volume of liquid to each tube that equals the "void volume" determined above.

The VOLUME IN WELL AT START does not include this "void volume". The "void volume" is excluded because it is below the artificial well bottom level, so the value you give for VOLUME IN WELL AT START reflects only the amount of liquid above the artificial well bottom level. So, for example if the "void volume" of a set of tubes was 100 μl and the tube contained 1700 μl in all, the VOLUME IN WELL AT START would be1600 μl.

File 70: Tip Height

The following example shows how to determine experimentally the two numbers required to edit a file so that a microtitration plate other than a standard microplate can be used.

1. Place a "24 across" template on the table.
2. Place the magazine in the back cavity of the template.
3. Accurately fill row P of a microtitration plate with 50 μl of water.
4. Place the plate in the middle cavity of the template.
5. Press "file", "70", and then "enter" to access File 70.
6. Press "start".
7. When TIP NOW AT "XXXX" is displayed, press "2800" and then "enter." This will bring the tips down to the plate.
8. Press the ↑ (yes) or ↓ (no) keys to raise or lower the tips until they just touch the meniscus of the water. Record the TIP NOW at number on a photocopy of the Tip Height Worksheet as the "50 ul Height."
9. Press the ↓ (no) key or enter numbers until the tips are just above the bottom of the wells. Record the TIP NOW at number on the worksheet as the WELL BOTTOM HEIGHT.
10. Press the "stop" key, and the display will read TIP HEIGHT COMPLETE FILE #70.
11. Press "file", the new file number that you want to use, and then "enter."

12 CALIBRATION PROCEDURES

12.1 Introduction

This section describes how to verify the accuracy and precision of the performance of the WellPro 384 Liquid Handler using a gravimetric procedure which utilizes the weight of water to determine the volume dispensed. While this method is the most accurate and controllable method, other methods could be used.

Alternative methods which could be used involve the transfer of:

- a solution of chromophore such as potassium dichromate and the determination of the absorbance.
- a solution of fluorescein or rhodamine and the determination of the fluorescence.
- a solution of a radioisotope and the determination of the radioactivity.

These methods are more difficult to perform and are subject to variations in detection; therefore they are to be considered secondary and are not recommended.

The frequency of calibration is dependent on the needs of your facility and is based on parameters such as the types of samples used, how frequently the WellPro liquid handler is used, and the maintenance program you have instituted.

When you are using a new WellPro liquid handler you should test eight samples from each tip to determine precision and accuracy. Subsequent verification can be carried out using the procedures in the "Quality Control" section (Section 12.6). We recommend that you maintain a log of calibration reports as this collected data will help you and WellPro Technical Service to identify any problems.

The WellPro Liquid Handler is factory calibrated and tested to insure the proper volume delivery. It is recommended that only qualified personnel be permitted to calibrate the instrument. If recalibration becomes necessary, you should call ProGroup Technical Service at 1-800-471-1916.

12.2 The Gravimetric Method

The gravimetric method of verification involves weighing each individual well containing water dispensed from the WellPro Liquid Handler. The analytical balance used in the procedure should be well-maintained, calibrated with an appropriate set of National Institute of Science and Technology certified weights (NIST Class S Weights; NBS Handbook 105-1, Circular 547; or equivalent), and should be capable of reading 0.001 g ± 0.0003 g. The gravimetric method also requires a thermometer which should be calibrated and readable to 0.5°C.

CAUTION: Since it is possible that water which has been added to the wells may evaporate during the calibration, we strongly recommend that you perform this procedure by dispensing water into one row at a time, then weighing the amount of water added to each well. (A row of 24 wells typically takes 10 minutes to weigh). Repeat this procedure for each subsequent row until all sixteen rows have been weighed.

Note: The WellPro liquid handler has been calibrated in the operational range of 20-26°C (68-79°F). Operation outside this range may affect the volumetric calibration and the system should be.calibrated if you intend to work outside the normal operating range.

If desired, you can make a volume correction by modifying the volume settings entered into the WellPro liquid handler when creating a "file". For example, if the operating temperature is 35°C and 51 µL are dispensed when 50 µL is anticipated, you may enter 49 µL into the file to compensate for the change.

If you will be continually working outside the specified temperature range, a permanent change can be made to make the volume correction automatically. For details, call ProGroup Technical Service at 1-800-471-1916.

12.2.1 Materials Required

To perform a gravimetric verification you will need the following:

1. Analytical Balance
2. Calibrated Thermometer
3. Calibration Weighing Wells
4. Calibration Plate
5. Forceps
6. Water (high purity)
7. New WellPro Tip Magazine and Tips
8. Photocopy of the Gravimetric Worksheet From This Manual (Table 12-2)

12.3 Calibration Procedure

1. Assemble all items and allow them to equilibrate at room temperature for at least one hour. During this time, use File 70 to determine the well bottom and 200 µL heights for the calibration plate. A detailed discussion of this procedure is presented in Chapter 10.

2. Weigh each well of the calibration plate. Record this and all other data on the worksheet.

3. Place a "24 across" template on the table.

4. Place a magazine of tips in the back cavity of the template.

5. Manually pipette 50 µl of water into each of the 384 wells of plate 26.Place plate 2 in the middle cavity of the template.

6. Place the calibration plate containing the pre-weighed wells in the front cavity of the template.

7. Access File 63 by pressing File, 63 and Enter.

8. Edit File 63 as described below:

Display Before Editing	Keystrokes	Display During Review
PLATE TO PLATE STEP TO EDIT FILE#63	STEP	
TRANSFER DIRECTION P2 TO P1 - P1 TO P2	STEP	TRANSFER DIRECTION P2 TO P1 - P1 TO P2
VOL IN WELL PLATE 1 (0-50) 0 uL	STEP	VOL IN WELL PLATE 1 (0-50) 0 UL
VOL IN WELL PLATE 2 (0-50) 50 UL	STEP (0-50)	VOL IN WELL PLATE 2 50 UL
TRANSFER MODE 1 TO 1 - 1 TO SEVERAL	STEP	TRANSFER MODE 1 TO 1 - 1 TO SEVERAL
TRANSFER VOLUME (0-50) 50 UL	40, ENTER	TRANSFER VOLUME (0-100) 40 UL
MIXES BEFORE TRANSFER (0-9) 0	STEP	MIXES BEFORE TRANSFER (0-9) 0
MIXES AFTER TRANSFER (0-9) 0	STEP	MIXES AFTER TRANSFER (0-9) 0
CHANGE TIPS? YES-NO	STEP	CHANGE TIPS? YES-NO
PLATE 1 TYPE FLAT-U-V-HEIGHT-TUBE	STEP	PLATE 1 TYPE FLAT-U-V-HEIGHT-TUBE
PLATE 2 TYPE FLAT-U-V-HEIGHT-TUBE	STEP	PLATE 2 TYPE FLAT-U-V-HEIGHT-TUBE
TRANSFER ROWS A-B-C-D-E-F-G-H I-J-K-L-M-N-O-P	STEP	TRANSFER ROWS A-B-C-D-E-F-G-H I-J-K-L-M-N-O-P
LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW	STEP	LIQ HANDLING SPEEDS RAPID-MEDIUM-SLOW
LINK TO STORED FILE (0-45, 0 = STOP) 0	STEP	LINK TO STORED FILE (0-45, 0 = STOP) 0
END OF FILE	ENTER	–
RUN-STORE		
PLATE TO PLATE START TO RUN FILE #63	START	–

9. Quickly weigh each well of the row dispensed.

10. Repeat steps eight through ten for each row.

11. Subtract the initial weighing from the final weighing for each well.

12.4 Calculations

To calculate the accuracy and precision of the WellPro liquid handler:

1. Determine the arithmetic mean (average) weight of each column by adding all sample (net) weights together and dividing by the number of samples.

2. Calculate the mean volume at the determined water temperature using the mean weight and Volume Conversion Factor (Table 12-1) using equation 12-1:

Volume = Weight x Volume Conversion Factor **12-1**

Table 12.1 Volume Conversion Factor

Water Temperature °C	Conversion Factor
15.0	1.002
17.5	1.002
20.0	1.003
22.5	1.003
25.0	1.004
27.5	1.005
30.0	1.005

3. Determine the standard deviation (SD) of the individual volumes within each column using the equation 11-2:

$$SD = \frac{\Sigma | Wi-WI^2}{N-1} \times \frac{1}{\text{density}} - \frac{\Sigma | Vi-VI^2}{N-1} \text{ Volume Conversion Factor} \qquad 12\text{-}2$$

Where: **Wi** is the weight of an individual well
WI is the average of the weight of the individual wells
Vi is the volume of an individual well
VI is the average of the volume of the individual wells
N is the number of wells (24)

4. Determine the coefficient of variation (CV) using the equation 12-3.

$$CV = \frac{SD \times 100\%}{\text{Mean Volume}} \qquad 12\text{-}3$$

A Gravimetric Worksheet presented as Table 12-2 can be used to assist in calculating the CV. (Four columns are shown for the sake of clarity. This worksheet should contain 12 columns).

TABLE 12-2 Gravimetric Worksheet

Date:_____
Tech:_____
Temperature:_____

GRAVIMETRIC WORKSHEET

		1			2			3			4	
ROW	IW *	FW*	NW*	IW	FW	NW	IW	FW	NW	IW	FW	NW
A	___	___	___	___	___	___	___	___	___	___	___	___
B	___	___	___	___	___	___	___	___	___	___	___	___
C	___	___	___	___	___	___	___	___	___	___	___	___
D	___	___	___	___	___	___	___	___	___	___	___	___
E	___	___	___	___	___	___	___	___	___	___	___	___
F	___	___	___	___	___	___	___	___	___	___	___	___
G	___	___	___	___	___	___	___	___	___	___	___	___
H	___	___	___	___	___	___	___	___	___	___	___	___
W			___			___			___			___
Vol			___			___			___			___
SD			___			___			___			___
CV			___			___			___			___

*IW = Initial Weight, FW = Final Weight, NW = Net Weight

12.5 Calibration of Non-Aqueous or Viscous Solutions

12.5.1 Introduction

The WellPro is normally calibrated with water as it is the most common liquid used in clinical and biological applications. Water has unique viscosity, surface tension, and binding characteristics. If you are using a different solvent; it is probable that these characteristics will differ radically from that of water, and the calibration using water might not be valid. Alternatively, if you are pipetting viscous fluids, the procedure described below should be used to calibrate the system.

☞ **Note: Many solutions, such as 20% glycerol, exhibit radical viscosity changes when cooled.**

12.5.2 Procedure

1. Determine the weight of a specific volume of the test solution at the temperature you plan to use routinely by weighing a known volume in a volumetric flask.

2. Calculate the density of the solution using the following formula:

$$d = \frac{\text{weight}}{\text{volume}}$$

3. Perform a shortened version of the "Calibration Procedure" at the temperature and with the file sequence you plan to use routinely. Weigh the wells of Row B at this same temperature.

4. Calculate the arithmetic mean (average) weight (W) by adding all sample weights together and dividing by the number of samples (24).

5. Calculate the volume dispense by dividing the mean weight dispensed by the density.

6. Calculate the standard deviation (SD) of the individual volumes using equation 12-4.

$$SD = \sqrt{\frac{\Sigma|Wi-Wl|^2}{23}} \times \frac{1}{\text{density}} = \frac{\Sigma|Vi-Vl|^2}{23} \qquad \text{12-4}$$

7. Calculate the coefficient of variation (CV) using equation 12-5:

$$CV = \frac{SD \times 100\%}{\text{mean volume}} \qquad \text{12-5}$$

8. If desired, a volume correction can be made by modifying the volume setting entered into WellPro liquid handler when creating a "File." for example, if 46 µL is dispensed for a viscous solution when 50 µL are anticipated, enter 54 µL (50 x 50/46) into the WellPro controller to compensate for the change.

The Non-Aqueous or Viscous Solution Worksheet presented as Table 12-3 can be used to assist in calculating the coefficient of variation. This worksheet can be reproduced as desired for calibration activities.

Table 12.3 Non-Aqueous or Viscous Solutions

Date:_____
Solution:_____
Temperature:_____
Tech:_____

Density:

Volume Weighed: _____ D = Density_____ $\frac{\text{Weight}}{\text{Volume}}$ =_____

Weight: _____

WellPro Weighing:

	Row B
1	_____
2	_____
3	_____
4	_____
5	_____
6	_____
7	_____
8	_____
9	_____
10	_____
11	_____
12	_____

W = Mean Weight = $\frac{W}{24}$ = _____

Volume Dispensed = $\frac{W}{D}$ = _____

Standard Deviation = $\frac{\overline{IVi} - VI^2}{23}$

Coefficient of Variation = $\frac{SD \times 100\%}{W}$ = _____

Calibration Setting = _____

12.6 Quality Control Procedures

Note: All laboratories should have a functioning quality control program to provide verification of the accuracy and precision of its test results.

The use of assayed control samples is helpful in monitoring the overall performance of the WellPro liquid handler and the reagents used in an assay. For example, when serum samples are studied, it is a good practice to assay serum controls in the normal and abnormal ranges with each batch of samples. When using commercial control sera, the value of each control should fall within the manufacturer's stated acceptable range for the analyte.

When a commercial control is not available, a control can be generated by adding known amounts of the analyte to a normal serum pool. For research specimens add known amounts of analyte to the appropriate matrix. Aliquot into containers and store at a temperature that is optimum for the sample's stability. For maximal control choose at least three levels of concentration: low, mid-range, and high. You may wish to choose additional concentrations at decision points or at questionable points on the standard curve where necessary.

A Levey-Jennings control chart, as shown in Figure 12.1, provides the simplest way to present daily control data. The chart is a sheet of rectangular coordinate graph paper marked with days on the horizonal axis and assay units on the vertical axis. The mean value, as well as the "limits of acceptable error", are indicated on the chart and are based on past laboratory experience (typically, mean ± 2 standard deviations). Plot the daily value on the control chart so you can easily see any trends or any point falling beyond the "limits of acceptable error."

Figure 12.1 Levey-Jennings Control Chart

12.6.1 Verification

Verification of the WellPro liquid handler calibration without the contribution of the reagents can also be part of the quality control system in your laboratory. Weigh only Row A wells or a "T" of wells consisting of Row A and Column 6, as illustrated in Figure 12.2, and construct a Levey-Jennings control chart using the mean (average) values for the Row and for the Column.

13 OPERATING HINTS AND MAINTENANCE

13.1 Overview

The WellPro 384 Liquid Handler is designed to be used with a minimum of maintenance. In this chapter, we describe routine operations that can be performed by the operator

CAUTION: Do not open the "Backpack Electronics" or disassemble the controller unit. There are no operator serviceable components in the instrument other than those describe in the *SERVICE AND MAINTENANCE and OPERATING INSTRUCTIONS* sections.

WARNING: For protection from injury by the WellPro liquid handler moving parts, *Do Not Remove* the blue plastic cover while the WellPro liquid handler is operating or the power is on.

13.2 Tips

The WellPro liquid handler has been calibrated using WellPro 384 disposable tips. The design of the tips and the manufacture of their internal volumes, orifice diameters, lengths, etc. to tight tolerance limits, assure the accuracy and precision of the liquid handler system. The length of the tips is of particular importance, since the proper "draw-off" of liquid from the tip depends heavily on its depth in the liquid that it is in contact with. Tips not manufactured by ProGroup may decrease the accuracy and precision of the system.

13.3 Power Interruptions

If the power supplied to the WellPro has been interrupted, the following actions will happen when the power is restored:

1. If you were editing a file, you will lose the file and will have to re-edit the file.

2. If you had just stored a file, the WellPro will store the file.

3. If you are in the middle of a run, you will lose the run. Homing the instrument will sequence it back to a point where you can start over.

13.4 Maintenance

13.4.1 Preventive Maintenance

To ensure trouble-free operation, keep the WellPro liquid handler clean and free of chemicals, dirt, and dust. To ensure trouble-free operation, you should keep your WellPro liquid handler clean and free of chemicals, dirt, and dust. Any liquids spilled on the WellPro liquid handler should be wiped off immediately.

Keep your WellPro liquid handler free of any corrosive reagents. Wipe spills immediately. Clean non contaminated work surfaces regularly with a damp cloth

13.4.2 Cleaning up Spills

If solution is accidentally spilled on the instrument, clean them up immediately. Although all electrical components within the instrument have been either shielded or sealed against fluid contact, there may be long-term deleterious effects from corrosion or salt accumulation. To ensure maximal safety and trouble-free operation, clean up spills immediately. Turn off and unplug the WellPro liquid handler before wiping up spills

13.4.3 Piston Plungers

On a periodic basis, gently apply a light silicone grease to the piston plungers of the 24-channel liquid head using a clean cloth or lint-free towel. This will keep them sealing well and inhibit corrosion.

13.4.4 Table Rails

The rails on which the WellPro table runs must be kept clean and free of foreign matter to ensure smooth operation. Periodically clean these by wiping with a clean cloth. **ALWAYS BE CERTAIN POWER IS TURNED "OFF" BEFORE CLEANING THE TABLE RAILS.**

13.4.5 Cleaning the Plastic Cover

The plastic covers should be cleaned using a dilute solution of a mild, nonabrasive cleaner, and then rinsed with a soft clean damp cloth.

Note: Do not use organic solvents that may damage the cover.

Decontamination may be performed by chemical means. Chlorine bleach is an acceptable decontamination solution. Ethanol and gluteraldehyde **should not** be used on the plastic surfaces. **DO NOT AUTOCLAVE.**

13.4.6 Fuse Replacement

Turn the power switch off and disconnect the power cord from the power outlet before replacing the fuse. This procedure will insure against electrical shocks.

For continued protection against electrical overload and fire hazard, replace the fuse only with one of the same type and rating (3 amps, 250V, Normal blow.)

13.5 Troubleshooting

If you encounter difficulties in the use of the instrument, please contact your local ProGroup Technical Service Department. A technical service representative may be able to resolve the problem over the telephone using a basic check-out procedures. If the problem cannot be resolved in this manner, the technical service representative will arrange for a field service representative to visit your lab to repair the unit. If the unit cannot be repaired in your lab, arrangements will be made to return the unit to ProGroup for factory repair

Only qualified personnel should attempt service or repair work on the WellPro operating circuits or mechanism.

14 WELLPRO WP384 SPECIFICATIONS

Dimensions:

	Controller	Liquid-Unit
Height:	2 ½"	16"
Width:	6 ¼"	12"
Depth:	6 ¾"	28 ½"

Weight: 70 lbs. (30.2kg)

Power Requirements: 120/220/240 VAC, 47-63 Hz, 2 amps at 120 VAC, 1 amp at 240 VAC.

Fuse: 3 amps, 250V, Normal blow

Room Temperature: 23± 3°C (68-79°F).

Volume Range: 0 to 50 µl programmable in 1 µl increments.

Accuracy: Typically 50 µl ± .5 µl, 10 µl ± .1 µl with phosphate buffered saline at 23°C.

Precision: Single and multi-channel head; typical coefficient of variation: less than ± 1% at 10 ul with deionized water at 23°C.

Time Delay: Programmable from 1 second to 999 minutes in 1 second steps. Accurate to 1 part in 1000 or better over ambient temperature range.

384 Well Microtitration Plates: 384-well (24 X 16), 5" X 3 ¼" microtitration plates typically used.